PHYSIOLOGICAL ANALYSIS OF THE CORTICAL RESPONSE OF THE SEA URCHIN EGG TO STIMULATING REAGENTS.

I. RESPONSE TO SODIUM CHOLEINATE AND WASP-VENOM

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The importance of the cortical response of sea urchin eggs in the process of fertilization or artificial activation has long been recognized and attention has been devoted to this problem. Loeb (1913) stated that the initiation of development in the sea urchin egg is due to a change in the surface of the egg. He maintained the theory, also, that cytolysis of the cortical layer generally results in membrane formation. According to Just (1919), some cortical change, beginning at the point of sperm-entry, sweeps over the egg before the actual elevation of the fertilization membrane. Gray (1922) postulated that the essential change of the egg surface is complete before any visible change is possible. Moser (1939) has found that after fertilization, the cortical granules embedded in the cortex of the Arbacia egg start to break down in a wave-like fashion and cause the formation of the fertilization membrane. He suggests that the breakdown of the cortical granules follows a preliminary release of calcium which may travel in a rapid wave-like fashion around the egg cortex. More recently, many interesting facts as to fertilization and activation are being found by the investigators of Runnström's institute.

However, despite great advances in our knowledge on this problem, it cannot yet be said that we have any sufficient understanding of the process of fertilization and activation. The present paper is the first of a series presenting the results of experiments which have been made in the hope of analyzing further the cortical response of the sea urchin egg.

MATERIAL AND METHODS

The sea urchin chosen for these experiments was *Hemicentrotus* (*Strongylocentrotus*) *pulcherrimus,* the spawning season of which extends over the whole winter. This species was used because the cortical granules of the egg are easily visible in the living state without centrifuging. With regard to the cortical granules of this species, Motomura (1941) studied them in detail, designating them "Janus green granules."

The eggs were secured in the usual manner. In all experiments only such egg material as showed good fertilizability in control experiments was used. For the first series of experiments a 1 per cent solution of sodium choleinate (Grübler) was made with sea water, sufficient amounts being added to sea water to make the desired solutions. The stock solution became ineffective in a few days but could be used for at least two days at room temperature (10–13° C.). The pH of the sodium choleinate solution was 8.1–8.3. For the second series of experiments, wasp-venom was obtained from *Polistes fadwigae.* Cutting the abdomen of the wasp, the poison gland was taken out and dried in a desiccator. At the time of an experiment, it was placed in a few drops of sea water and venom was extracted. The concentration of wasp-venom was difficult to determine. All the experiments were made at room temperature.

EXPERIMENTS WITH SODIUM CHOLEINATE

First, some typical experiments in which sodium choleinate was used to induce the formation of the fertilization membrane will be reported. Unfertilized eggs were put into solutions of sodium choleinate of various concentrations and their response was observed. It was found that sodium choleinate was effective in inducing the formation of the fertilization membrane, and the mode of response of the eggs was somewhat different from that obtained in experiments with butyric acid or urea solution. One of the results of the experiments is shown in Table I.

TABLE I

Formation of the fertilization membrane by means of sodium choleinate
(temp. 11° C.)

Number of drops of Na-choleinate solution added to 1 cc. of sea water	Mode of membrane formation
20	The membrane is violently separated in 10 seconds (Fig. 1, a).
15	The membrane is violently separated in 40 seconds (Fig. 1, a).
10	"Normal" membrane formation took place in 50% of the eggs within 2 minutes, while some eggs formed only partially (Fig. 1, b, c). After 3 minutes, 92% of the eggs had completed membrane elevation.
5	No response was observed in most of the eggs after 5 minutes. Partial membrane formation occurred in a few eggs (Fig. 1, c).
4	No response was observed in any of the eggs after 5 minutes.

At the optimum concentration of sodium choleinate, formation of a normal-appearing fertilization membrane occurred within a few minutes. However, the mode of membrane elevation was rather different from that which takes place when the eggs are treated with butyric acid or urea solution. When such eggs are returned to sea water, roughening of the whole surface takes place within several seconds. But in the experiments with sodium choleinate, roughening of the whole surface of the eggs did not occur before membrane elevation. The membrane began to rise in one or several places, and a few minutes were required to complete its full elevation.

Too high concentration of sodium choleinate resulted in violent separation of the membrane and subsequent cytolysis. When a solution of sodium choleinate of too low concentration was used, no response was observed in most of the eggs, but partial separation of the membrane sometimes took place in a few eggs. Such par-

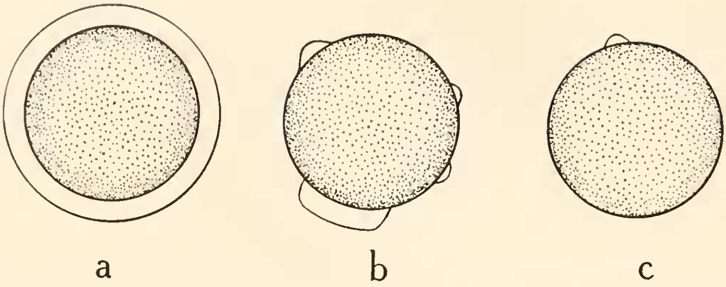

FIGURE 1. Eggs treated with sodium choleinate. a, an egg with completely elevated membrane; b and c, eggs with partially elevated membranes.

tially separated membranes remained as such even after twenty minutes' immersion in the solution.

In the next experiments, eggs treated with sodium choleinate solution of the optimum concentration (1 cc. of sea water plus 10 drops of 1% sodium choleinate solution) were washed with normal sea water when a partial separation of the membrane had just taken place. It was found that in such eggs membrane separation stopped and no further progress of the cortical change was observed. Upon examining the eggs with the oil immersion objective, it became obvious that the breakdown of the cortical granules occurred only in the part where the membrane had separated and the granules were intact elsewhere (Fig. 2). It was also found that when such eggs were inseminated after several washings with sea water, fertilization took place and the membrane separated all over the egg surface within a few minutes. The cortical granules of these eggs were thoroughly broken down. Most of these eggs, however, showed polyspermic development.

When unfertilized eggs are insufficiently treated with butyric acid, the fertilization membrane is sometimes elevated eccentrically or very slightly, but such a

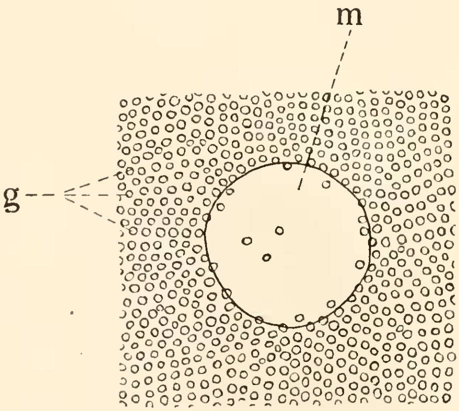

FIGURE 2. The cortex of the egg with partially elevated membrane. The cortical granules are broken down only in the area where the membrane is elevated. g, cortical granules; m, partially elevated membrane.

strictly partial separation of the membrane as in the case of sodium choleinate seldom occurs. It is interesting that the cortical response to an insufficient treatment with sodium choleinate is somewhat different from that to an insufficient treatment with butyric acid.

Experiments with Wasp-venom

Öhman (1944) reported that a membrane similar to a normal fertilization membrane was formed under the action of bee-venom. The experiments with bee-venom have thereafter been repeated and extended by Runnström (1947), and interesting results, especially concerning the behavior of the cortical material, have been obtained.

The present author has found that there is something in common between the actions of wasp-venom and sodium choleinate. If the unfertilized eggs of *Hemicentrotus pulcherrimus* are exposed to the action of appropriate concentrations of wasp-venom in sea water, small blisters appear at several places on the egg surface and soon an elevation of the membrane begins. If the action of the wasp-venom is continued longer, a complete membrane is formed in a few minutes. However, if the eggs are washed with sea water when an elevation of the membrane has occurred only locally and not over the whole egg surface, the membrane formation is interrupted as in the experiments with sodium choleinate. If these are observed with the oil immersion objective, it is found that the cortical granules have been broken down in the part of the egg cortex where the membrane is separated, while they are intact in the remaining areas.

Runnström has reported that when the treatment with bee-venom was interrupted by washing with sea water the membrane formation in Psammechinus eggs took place almost to the same extent as under continuous action of the bee-venom. The same results were obtained in Hemicentrotus eggs also, when a high concentration of wasp-venom was used. But a partial elevation of the membrane took place when an appropriate concentration of wasp-venom was used.

Discussion

It is well known that the first remarkable change of the sea urchin egg at the time of fertilization is, in general, a roughening process at the surface. Moser (1939) found in Arbacia eggs that this phenomenon is a consequence of the breakdown of the cortical granules. He found also that in from 10 to 20 seconds after insemination the cortical granules start to break down in a wave-like fashion, beginning at the site of sperm-entry and ending at the opposite pole of the egg. A similar behavior of the cortical granules is also observed in Hemicentrotus eggs (Motomura, 1941).

It has been suggested by several workers that some sort of invisible change is complete before any visible change occurs at the time of fertilization. This suggestion has generally been made on the basis of the fact that the block to polyspermy is rapidly established with the attachment or entry of one spermatozoon. According to Just, for instance, the "wave of negativity" progressively sweeps over the egg from the point of sperm-entry, preceding the actual beginning of membrane lifting, so that before the membrane begins to lift sperm can no longer enter at any point of the egg surface. It might be postulated that the breakdown of the cortical granules follows the so-called wave of negativity. But there has been no evidence to prove causality between them. There may be some possibility that the wave of negativity is not connected with the breakdown of the cortical granules.

If we assume that the breakdown of one cortical granule does not automatically induce the breakdown of its neighbours, then the granule breakdown must be the consequence of a more profound cortical change of a self-propagating nature. The results presented in this paper make it possible to discuss some problems on the process of the breakdown of the cortical granules. If eggs treated with sodium choleinate or wasp-venom are washed with sea water when elevation of the membrane has occurred only at several places on the egg surface, the membrane formation is interrupted. It has been noted that the cortical granules are broken down in the parts of the egg cortex from which the membrane is separated, while they are intact in the remaining areas. It is evident that the failure of the granular breakdown is not due to some impairing effect of the reagents, because, as stated above, the remaining granules are broken down if insemination is carried out afterwards. These facts constitute the evidence for concluding that the breakdown of the cortical granules itself at any point does not become the cause of the breakdown of the neighbouring granules. It seems quite possible, therefore, that some invisible cortical change of a propagating nature must occur in order that the cortical granules are broken down at the time of fertilization. Whether such an invisible cortical change is identical with Just's "wave of negativity" or not, is still obscure, because there has been no evidence to indicate that the mechanism to exclude supernumerary spermatozoa is the cause of breakdown of the cortical granules.

The work of Yamamoto (1944a, 1944b) on the eggs of the fish, *Oryzias latipes,* has demonstrated that the first visible change at fertilization is the wave-like breakdown of alveoli which are embedded in the cortical protoplasm of ripe unfertilized eggs. He postulated that a reaction of some kind, or an impulse, may be provoked by the sperm and conducted in a wave-like fashion, causing such alveolar breakdown. He termed this invisible wave the "fertilization-wave." The Oryzias egg is much larger than the sea urchin egg and its structure and nature differ from those of the sea urchin egg to a considerable degree. But the processes of the fertilization reaction in the two animals seem to have something in common with each other. The invisible cortical change in the sea urchin egg resembles the "fertilization-wave" in Oryzias eggs in its self-propagating nature, in its occurrence prior to the breakdown of the cortical structure and in its function of causing the breakdown of the cortical structure. Therefore, the author wishes to call the invisible cortical change in sea urchin eggs, also, the "wave of fertilization" or the "fertilization-wave."

As suggested elsewhere in this article, the cortical response to an insufficient treatment with sodium choleinate is somewhat different from that to an insufficient treatment with butyric acid. By means of sodium choleinate a partial separation of the membrane can be induced. This suggests that sodium choleinate causes the breakdown of the cortical granules without intervention of the fertilization-wave. This suggestion is also applicable to wasp-venom. On the other hand, such a strictly partial separation of the membrane as in the case of sodium choleinate seldom occurs as the result of an insufficient treatment with butyric acid. This probably indicates that butyric acid gives rise to the fertilization-wave, indirectly inducing the breakdown of the cortical granules. The data seem insufficient to warrant a more extended analysis, but indicate the desirability of a more refined approach to the problem of the response of the eggs to parthenogenetic reagents.

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SUMMARY

1. The formation of the fertilization membrane in Hemicentrotus eggs can be induced by an appropriate treatment with sodium choleinate or wasp-venom. When the concentration is optimum, the membrane begins to rise in one or several regions of the egg surface and several minutes are required to complete its full elevation.

2. If the eggs are washed with sea water when elevation of the membrane has occurred only locally and not over the whole egg surface, the membrane formation is interrupted. The cortical granules have been broken down only in the part of the egg cortex where the membrane is separated while they are intact in the remaining areas.

3. These results constitute the evidence for the conclusion that the breakdown of the cortical granules itself at any point does not become the cause of the breakdown of the neighbouring granules. It seems to follow, therefore, that some invisible cortical change of a propagating nature must occur in order that the cortical granules be broken down at the time of fertilization. This invisible cortical change may be called the "wave of fertilization" or the "fertilization-wave," since it resembles Yamamoto's "fertilization-wave" in Oryzias eggs.

4. It is suggested that sodium choleinate and wasp-venom cause the breakdown of the cortical granules without intervention of the fertilization-wave.

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